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Electrophoretic Evidence for Interspecific Hybridization in *Polystichum*

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Hybridization and polyploidy in *Polystichum* (Dryopteridaceae) have contributed to the morphological diversity and taxonomic complexity in this widespread genus. Patterns of reticulate evolution are particularly evident in the *Polystichum* complex from western North America (W. Wagner, 1973; D. Wagner, 1979), and several sterile interspecific hybrids have been reported (see P. Soltis et al., 1987, for review). As part of a comprehensive molecular analysis of polyploidy, its causes, and genetic consequences, we have studied two additional interspecific hybrids. In this paper we provide electrophoretic documentation of hybridization between *P. andersonii* and *P. munitum* and between *P. lemmonii* and *P. munitum*. Both hybrids were previously reported based on morphological and cytological evidence (W. Wagner, 1973).

Polystichum andersonii occupies lowland coastal forests in British Columbia and southeastern Alaska, montane forests in western Washington and Oregon, and also occurs disjunctly in southeastern British Columbia, northern Idaho, and northwestern Montana. This species is tetraploid ($2n = 164$; W. Wagner, 1973; D. Wagner, 1979), but its ancestry is uncertain. Warren Wagner (1973) suggested that this species is of autopolyploid origin based on its distinctive leaf morphology and the presence of a vegetative bud on the rachis of the frond. However, cytological data (W. Wagner, 1973) show that *P. andersonii* contains a chromosome complement homologous with the common diploid *P. munitum*, suggesting instead an allopolyploid origin for *P. andersonii*. Anatomical, chromatographic, and electrophoretic data also support this hypothesis (D. Wagner, 1979; P. Soltis et al., unpubl. data). Although *P. munitum* is a likely candidate for one of the diploid progenitors of *P. andersonii*, the identity of the other parental species is currently unknown, although D. Wagner (1979) described a specimen from northern British Columbia that seems to fit the predicted morphology of the second progenitor.

Polystichum lemmonii ($2n = 82$) occurs on open, serpentine, montane slopes in northern California, southwestern and central Oregon, and central Washington. Following D. Wagner (1979), *P. lemmonii* is herein considered distinct from the South American *P. mohrioides*.

Polystichum munitum ($2n = 82$) inhabits moist coniferous forests from California to southeastern Alaska, with disjunct populations in eastern Washington, northern Idaho, northwestern Montana, and northeastern Oregon. Populations of *P. munitum* are often very large, consisting of thousands of individuals. This species is highly outcrossing and experiences significant interpopulational gene flow (P. Soltis & D. Soltis, 1987).

Thirty to forty plants morphologically intermediate between *P. andersonii* and *P. munitum* were observed on Deer Peak near Ketchikan, Alaska. All mature

plants possessed the distinctive vegetative bud of *P. andersonii*, yet the fronds were less dissected than typical leaves of *P. andersonii*, suggesting the influence of *P. munitum* or another once-pinnate species. Because *P. munitum* occurs in this region, we hypothesized that these morphologically intermediate plants were hybrids between *P. andersonii* and *P. munitum*, although neither putative parental species was found in the immediate vicinity of the hybrid population.

A single plant morphologically intermediate between *P. lemmonii* and *P. munitum* was detected in the Beverly Creek drainage of Kittitas County, Washington. Fronds were comparable in size to those of *P. munitum*, yet showed evidence of dissection, similar to that observed in the bipinnate *P. lemmonii*. Both *P. lemmonii* and *P. munitum* occur in the Beverly Creek drainage, although they occupy different habitats. Thus it seemed likely that the unusual morphology resulted from hybridization between these two diploid species.

Materials and Methods

Plants.—Twenty-eight putative hybrids between *P. andersonii* and *P. munitum* were examined electrophoretically (see Table 1 for collection data). Individuals from four populations of *P. andersonii* were also examined. Individuals from three populations of *P. munitum* were analyzed alongside the putative *P. andersonii* × *munitum* hybrids, and additional data from several other populations were also used for comparisons (P. Soltis & D. Soltis, 1987; P. Soltis et al., unpubl. data). The single putative hybrid between *P. lemmonii* and *P. munitum* was compared electrophoretically to individuals of both parent species from the Beverly Creek drainage and to plants from other populations of both species (P. Soltis & D. Soltis, 1987; P. Soltis et al., unpubl. data). Leaf material was collected in the field and stored in plastic bags under refrigeration until electrophoresis was conducted. Collection data and sample sizes for all taxa are provided in Table 1. Vouchers were deposited at WS.

Table 1. Collection Data and Sample Sizes (*N*) for Populations of *Polystichum* Species and Hybrids Examined Electrophoretically.

P. andersonii.—**Alaska:** Juneau, West Glacier Trail, Mendenhall Glacier, *Soltis & Soltis 1785* (22). **Oregon:** Linn County, Pamelia Lake, Mt. Jefferson Wilderness, *Soltis & Soltis 1904* (7). **British Columbia, Canada:** 4 mi NE of Exchamsiks Provincial Park, *Soltis & Soltis 1768* (14); 2–3 mi E of Stewart on Hwy. 37A, *Soltis & Soltis 1800* (13).

P. lemmonii.—**Washington:** Kittitas County, Beverly Creek Trail, Wenatchee National Forest, *Soltis, Soltis, & Riley 1974* (34).

P. munitum.—**Alaska:** Revillagigedo Island, 5.5 mi S of Ketchikan, *Soltis & Soltis 1778* (28). **Idaho:** Benewah County, 3.1 mi SW of Emida along Hwy. 95, *Soltis & Soltis 1844* (5); N of St. Maries along Hwy. 5, *Soltis & Soltis 1845* (7). **Washington:** Kittitas County, Beverly Creek Trail, Wenatchee National Forest, *Soltis, Soltis, & Riley 1972* (1).

P. andersonii × *munitum*.—**Alaska:** Revillagigedo Island, Deer Peak Trail, Ketchikan, *Soltis & Soltis 1770* (28).

P. lemmonii × *munitum*.—**Washington:** Kittitas County, Beverly Creek Trail, Wenatchee National Forest, *Soltis, Soltis, & Riley 1977* (1).

Electrophoresis.—Electrophoretic procedures generally followed D. Soltis et al. (1983). Leaf tissue was prepared using the tris-HCl grinding buffer of D. Soltis et al. (1983); 12% PVP was used (wt/vol). Starch gel concentration was 12.5%.

Nine enzymes were examined: aspartate aminotransferase (AAT), NAD-dependent glyceraldehyde 3-phosphate dehydrogenase ([NAD]G3PDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6PGD), shikimate dehydrogenase (SkDH), and triosephosphate isomerase (TPI). AAT, LAP, PGI, and TPI were resolved on gel and electrode buffer system 8 of D. Soltis et al. (1983) as modified by P. Soltis et al. (1987). MDH, PGM, and SkDH were resolved on system 9 of D. Soltis et al. (1983); [NAD]G3PDH and 6PGD were resolved using system 1 (D. Soltis et al., 1983). Staining for all enzymes except LAP followed D. Soltis et al. (1983); staining for LAP followed D. Soltis and Rieseberg (1986).

The genetic control of the enzyme banding patterns was easily inferred based on the typical subunit structure and subcellular compartmentalization of the enzymes (Gottlieb, 1981, 1982). Isozymes were numbered sequentially, with the most anodally migrating isozyme designated *1*. Allozymes were denoted alphabetically, with the farthest migrating allozyme designated *a*.

Results

Polystichum andersonii × *munitum.*—Thirteen loci were interpreted: *Aat, G3pdh-1, G3pdh-2, Lap, Mdh-1, Pgi-2, Pgm-1, Pgm-2, 6pgd-2, Skdh, Tpi-1, Tpi-2*, and *Tpi-3*. These loci designations reflect an apparent gene duplication for TPI (P. Soltis & D. Soltis, unpubl. data). *Pgi-1* and *6pgd-1* were not clearly resolved in all samples and were therefore omitted from the analysis. All enzymes migrated anodally.

Polystichum andersonii and *P. munitum* shared alleles at all loci, although distinct differences in allele frequencies were observed for *Pgi-2* (Table 2). However, *P. andersonii* exhibited fixed heterozygosity at *Skdh* and a five-banded pattern in the more anodal zone of activity for TPI. It is unclear whether this five-banded pattern represents fixed heterozygosity at *Tpi-1* or *Tpi-2* or both of these loci. Although the parental taxa are similar allozymically, electrophoretic data support the hypothesized hybrid origin of the plants from Deer Peak. The putative hybrids clearly combined the genotypes of *P. andersonii* and *P. munitum* at *Pgi-2* (Fig. 1). All individuals of *P. andersonii* possessed *Pgi-2a*; this allele was in low frequency in *P. munitum*. The most common allele in *P. munitum* was *Pgi-2c*. The putative hybrids all displayed the heterozygous genotype *Pgi-2ac*, and most individuals clearly displayed unbalanced staining, with two doses of allele *a* and one of allele *c* (Fig. 1). Such dosage effects would be expected in a triploid hybrid between an allotetraploid and a diploid and have been reported for other triploid hybrids (e.g., P. Soltis et al., 1987). The hybrids also exhibited a fixed heterozygous pattern for *Skdh* identical to that of *P. andersonii* (Fig. 2) and the complex banding pattern of *P. andersonii* for TPI. The

TABLE 2. Allele Frequencies in Populations of *Polystichum andersonii*, *P. lemmonii*, and *P. munitum* Included in this Study. Population numbers correspond to those in Table 1.

	P. andersonii Population				*P. lemmonii* Population	*P. munitum* Population			
Locus/Allele	1785	1904	1768	1800	1974	1844	1845	1778	1972
Aat									
a	1.0	1.0	1.0	1.0	0.985	1.0	1.0	1.0	1.0
b	0.0	0.0	0.0	0.0	0.015	0.0	0.0	0.0	0.0
G3pdh-1									
a	1.0	—	1.0	—	1.0	1.0	1.0	1.0	—
G3pdh-2									
a	0.0	—	0.0	—	1.0	0.0	0.0	0.0	0.0
b	1.0	—	1.0	—	0.0	1.0	1.0	1.0	1.0
Lap									
a	0.0	0.0	0.0	0.0	0.0	—	—	0.240	—
b	0.0	0.0	0.0	0.0	0.882	—	—	0.0	—
c	1.0	1.0	1.0	1.0	0.118	—	—	0.760	—
Mdh-1									
a	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.036	0.0
b	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.964	1.0
Pgi-2									
a	1.0	1.0	1.0	1.0	0.971	0.100	0.071	0.111	0.0
b	0.0	0.0	0.0	0.0	0.015	0.0	0.0	0.0	0.0
c	0.0	0.0	0.0	0.0	0.015	0.800	0.928	0.648	1.0
d	0.0	0.0	0.0	0.0	0.0	0.100	0.0	0.148	0.0
e	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.092	0.0
Pgm-1									
a	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.940	1.0
b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.060	0.0
Pgm-2									
a	0.0	0.0	0.0	0.0	1.0	0.100	0.143	0.020	0.500
b	1.0	1.0	1.0	1.0	0.0	0.900	0.857	0.980	0.500
6pgd-2									
a	0.0	0.0	0.0	—	0.129	0.100	0.071	0.0	0.0
b	1.0	1.0	1.0	—	0.871	0.200	0.571	0.643	1.0
c	0.0	0.0	0.0	—	0.0	0.700	0.357	0.357	0.0
Skdh	*	*	*	*					
a					1.0	1.0	1.0	1.0	1.0
Tpi-1									
a	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Tpi-2	*	*	*	*					
a					0.939	0.0	0.0	0.0	0.0
b					0.061	1.0	1.0	1.0	1.0
Tpi-3									
a	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

*denotes fixed heterozygous patterns.
—indicates no data available.

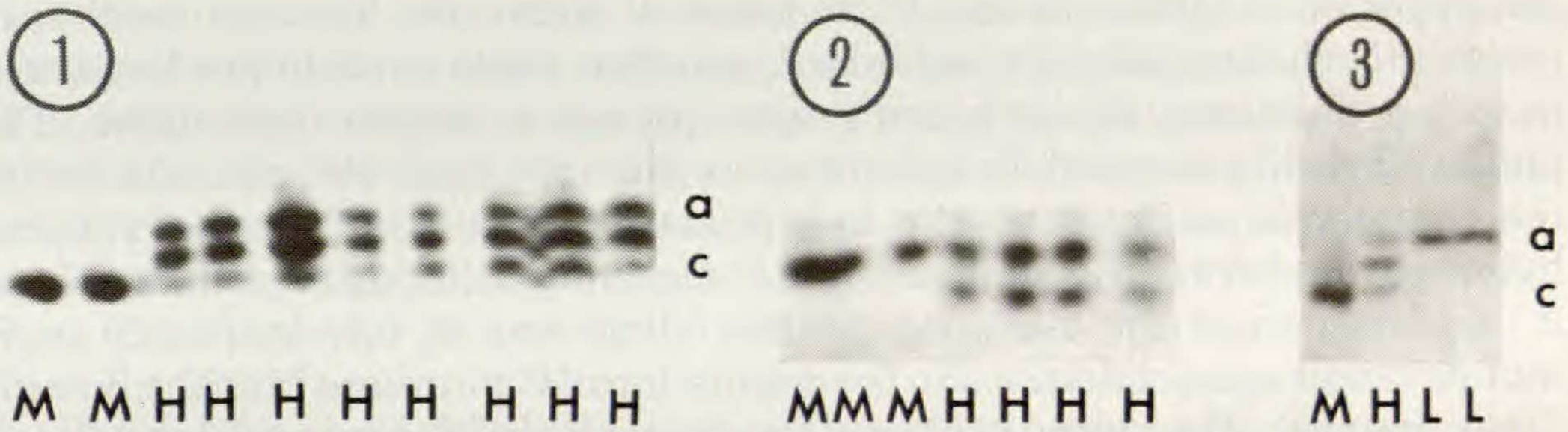

FIG. 1–3. Starch gels of *Polystichum*. In Figs. 1 and 3, numbers to the right of photographs designate allozymes. In all figures, letters below photographs designate species: L = *P. lemmonii*, M = *P. munitum*, H = hybrid. FIG. 1. PGI in *P. munitum* and *P. andersonii* × *munitum*. *Polystichum andersonii* (not pictured) expresses allele *a*. Note unbalanced staining in hybrids. FIG. 2. SkDH in *P. munitum* and *P. andersonii* × *munitum*. Hybrids possess the two-banded fixed heterozygous pattern of *P. andersonii* (not shown). FIG. 3. PGI in *P. lemmonii*, *P. munitum*, and their hybrid.

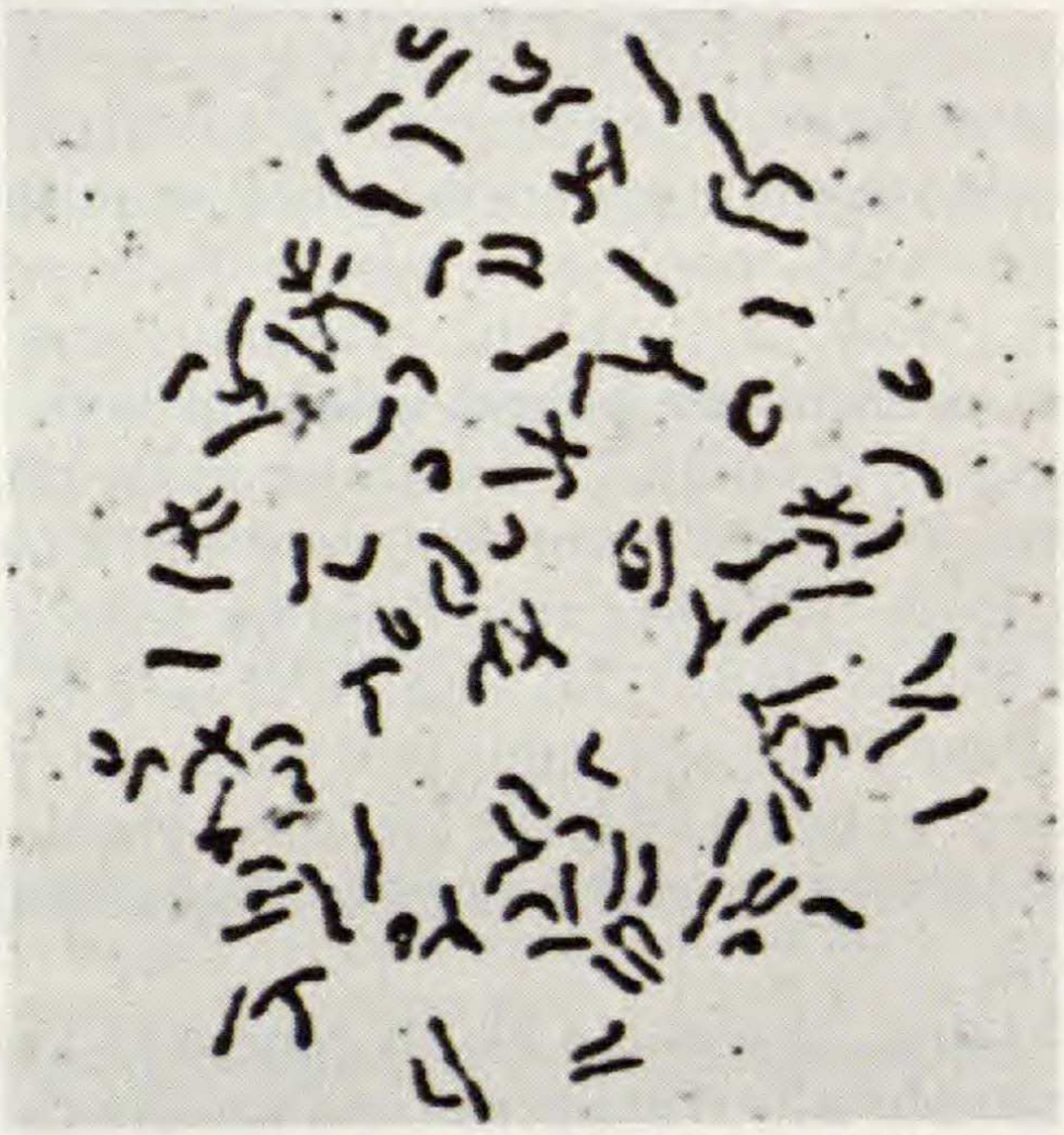

FIG. 4. Mitotic chromosome squash of triploid *P. andersonii* × *munitum* (*Soltis & Soltis 1770*). $2n = 123$. Magnification is 1,000×.

putative hybrids could not be distinguished from either parent at *Aat*, *G3pdh-1*, *G3pdh-2*, *Lap*, *Mdh-1*, *Pgm-1*, *Pgm-2*, *6pgd-2*, and *Tpi-3*.

Chromosome counts of the putative hybrids provide further evidence for their interspecific hybrid origin because all individuals were triploid, with $2n = 123$ (Fig. 4).

Polystichum lemmonii × *munitum*.—Eleven loci were interpreted: *Aat*, *G3pdh-2*, *Mdh-1*, *Pgi-2*, *Pgm-1*, *Pgm-2*, *6pgd-2*, *Skdh*, *Tpi-1*, *Tpi-2*, and *Tpi-3*. *G3pdh-1* and *Lap* were not clearly resolved in the putative hybrid.

Polystichum lemmonii and *P. munitum* differed in all populations examined for *G3pdh-2* and *Tpi-2* (Table 2). The putative hybrid clearly combined the

genotypes of *P. lemmonii* and *P. munitum* at these two loci. For *6pgd-2*, *P. lemmonii* exhibited alleles *a* and *b*. In *P. munitum* allele *a* was in low frequency in two populations; alleles *b* and *c* were present in higher frequencies. The putative hybrid possessed the heterozygous genotype *6pgd-2bc*, consistent with the hypothesis of interspecific hybridization. Data for PGI also support hybridization between *P. lemmonii* and *P. munitum*. Although *P. lemmonii* and *P. munitum* share the allele *Pgi-2a*, this allele was in low frequency in *P. munitum* and was not detected in the population of *P. munitum* from the Beverly Creek drainage. The hybrid exhibited *Pgi-2a* (typical of *P. lemmonii*) and *Pgi-2c* (typical of *P. munitum*), also consistent with the hypothesis of interspecific hybridization (Fig. 3). The parental species could not be differentiated consistently at any of the other loci. Such allozymic similarity between congeneric fern species is unusual (D. Soltis & P. Soltis, 1989) and may reflect relatively recent radiation in this species complex.

Discussion

Electrophoretic data provide clear documentation of interspecific hybridization between *Polystichum andersonii* and *P. munitum*, and between *P. lemmonii* and *P. munitum*. Morphological intermediacy, additivity at one or more enzyme loci, and a triploid chromosome count for *P. andersonii* × *munitum* confirm the hybrid origin of both putative hybrids. Although only a single *P. lemmonii* × *munitum* individual was observed, hybrids of *P. andersonii* and *P. munitum* were thriving and apparently reproducing on Deer Peak. Whether this resulted from sexual or asexual reproduction requires further study. The absence of both *P. andersonii* and *P. munitum* from the Deer Peak site coupled with the vitality of the *P. andersonii* × *munitum* population suggests that this hybrid was not formed on Deer Peak but originated elsewhere and is capable of spreading via either sexual or asexual reproduction.

Interspecific hybridization in western North American *Polystichum* is both taxonomically and geographically widespread and apparently occurs frequently when two species occur sympatrically. This suggests that prezygotic isolating barriers are not well established in this species complex. In fact, several factors may actually promote interspecific hybridization. All species of *Polystichum* analyzed to date, including polyploids, are outcrossing (P. Soltis & D. Soltis, 1987; P. Soltis et al., 1988; D. Soltis & P. Soltis, 1987). *Polystichum munitum* is highly outcrossing; rates of intragametophytic selfing range from 0 to 3% (P. Soltis & D. Soltis, 1987). *Polystichum lemmonii* is also outcrossing, with intragametophytic selfing estimates for six populations ranging from 0 to 18% (P. Soltis and D. Soltis, unpubl. data). Furthermore, *F*, the fixation index (Wright, 1965), indicates only slight deviation from Hardy-Weinberg genotypic expectations in all populations of *P. lemmonii* (P. Soltis and D. Soltis, unpubl. data). These data suggest that random mating among gametophytes occurs in populations of *P. lemmonii* and that *P. lemmonii* is outcrossing in the sense of seed plants. Similar analyses of *P. andersonii* have not been possible because no polymorphic loci were observed. The tendency for intergametophytic matings

coupled with poorly developed isolating mechanisms in *Polystichum* may in large part determine the high incidence of interspecific hybridization.

The frequency of interspecific hybridization in *Polystichum* may also have significant implications for the evolutionary history of the numerous allopolyploid species in *Polystichum*. It seems likely that these allopolyploids may be polyphyletic, having arisen several times throughout their geographic ranges. For example, *P. lemmonii* and either *P. munitum* (W. Wagner, 1973) or *P. imbricans* (D. Wagner, 1979) are considered the diploid progenitors of the allotetraploid *P. scopulinum* (W. Wagner, 1973; D. Wagner, 1979). The hybrid *P. lemmonii* × *munitum* documented herein occurs sympatrically with *P. lemmonii*, *P. munitum*, and scattered individuals of *P. scopulinum*. It is therefore possible that *P. scopulinum* from the Beverly Creek drainage of central Washington arose *in situ*. Other populations of *P. scopulinum* from throughout its range may also represent independent origins and different genotypes. Evidence for multiple origins of *Polystichum* allopolyploids is currently being sought using isozyme and DNA markers.

The significance of rampant hybridization in *Polystichum* extends beyond the production of typically sterile hybrids and taxonomic complexity. Frequent interspecific hybridization provides the opportunity for multiple allopolyploid events and may represent an important force in the history of allopolyploid species.

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